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## Response to Methylphenidate in Adults With ADHD is Associated With a Polymorphism in *SLC6A3* (*DAT1*)

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**In this pharmacogenetic study in adults with ADHD (n = 42), a stratified analysis was performed of the association between response to methylphenidate (MPH), assessed under double-blind conditions, and polymorphisms in the genes encoding the dopamine transporter, *SLC6A3* (*DAT1*), the norepinephrine transporter, *SLC6A2* (*NET*), and the dopamine receptor D4, *DRD4*. The VNTR polymorphism in the 3' untranslated region of *SLC6A3* was significantly associated with an increased likelihood of a response to MPH treatment (OR 3.8; 95% CI 1.0–15.2, and OR 5.4; 95% CI 1.4–21.9, depending on the definition of response) in carriers of a single 10-repeat allele compared to patients with the 10/10 genotype. The polymorphisms in *DRD4* and the *SLC6A2* were not associated with treatment response. This study supports a role of the *SLC6A3* genotype in determining the response to MPH in the treatment of adults with ADHD. © 2007 Wiley-Liss, Inc.**

**KEY WORDS:** adults; ADHD; methylphenidate; pharmacogenetics; medication response; dopamine transporter *SLC6A3* (*DAT1*)

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### INTRODUCTION

Attention deficit/hyperactivity disorder, or ADHD, is a neuropsychiatric disorder that affects between 3% and 6% of

children worldwide [Faraone et al., 2003]. Family studies [Cantwell, 1972; Biederman et al., 1992; Faraone et al., 2005] as well as twin studies [e.g., Levy et al., 1997; Rietveld et al., 2004] and adoption studies [Moore and Fombonne, 1999; Sprich et al., 2000] point to a strong genetic component in ADHD, with heritability estimates of 60% to 90% [Levy et al., 1997; Faraone et al., 2005]. Symptoms of the disorder persist into adulthood in more than 60% of cases and prevalence rates up to 5% in adults have been reported [Biederman et al., 2000; Barkley et al., 2002; Kooij et al., 2005; Faraone et al., 2006; Kessler et al., 2006]. Treatment with stimulants such as methylphenidate (MPH) and d-amphetamine is effective in about 70% of children and in 25–78% of adults, depending on patient samples, doses, and outcome measures used [Spencer et al., 1996; Faraone et al., 2004].

Several studies have implicated genetics not only in ADHD susceptibility, but also in the response to medication. A pilot study of our own group in 28 sib pairs with MPH-treated ADHD suggested familial clustering of MPH response based on a significant correlation of MPH response between siblings [van der Meulen et al., 2005]. A number of genes thought to be associated with an increased ADHD risk have been tested for their association with the response to MPH in childhood ADHD [for reviews also see References McGough, 2005; Polanczyk et al., 2005; Khan and Faraone, 2006]. Most studies have evaluated the *SLC6A3* gene, which encodes a direct target of MPH action, the dopamine transporter DAT1. Studies have concentrated on a variable number of tandem repeat polymorphism (VNTR) in the 3' untranslated region of this gene, which appears to be associated with ADHD [Cook et al., 1995; Waldman et al., 1998; Daly et al., 1999; Curran et al., 2001], although recent meta-analyses results are contradictory and suggest heterogeneity in the findings [Li et al., 2006; Yang et al., 2007]. The studies regarding the involvement of this variant in MPH treatment outcome have yielded rather inconsistent results. Winsberg and Comings [1999] were the first to report a poorer response to MPH in patients homozygous for the 10 repeat allele (the ADHD “risk allele”) in a prospective study of 30 ADHD children. Roman et al. [2002] replicated these findings in their prospective study of 50 children with ADHD, as did Cheon et al. [2005] in their study of 11 children. Furthermore, a recent study in 16 children with ADHD investigating the effect of single-dose treatment (with MPH or atomoxetine) on short interval cortical inhibition (SICI) also found significant effects of *SLC6A3* genotype, with increasing SICI in heterozygotes for the 10-repeat allele, but decreasing SICI in homozygotes [Gilbert et al., 2006]. SICI is reduced in ADHD patients compared to controls and is a possible neurophysiological marker of behavioral symptoms in ADHD [Moll et al., 2000]. Kirley et al. [2003] on the other hand, found that an increased response to MPH was associated

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with transmission of the 10-repeat allele in their retrospective, family-controlled association study that included 119 children with ADHD. Evidence that it is the 9-repeat allele rather than the 10-repeat allele that is associated with impaired response to MPH was provided by Stein et al. [2002]: the researchers found that homozygotes for the 9-repeat allele had an impaired response to MPH in a prospective study of 47 children [Stein et al., 2002]. As the authors stated, this association had also been found in an extended analysis of the sample reported earlier by Kirley et al. [referenced in Stein et al., 2005]. Interestingly, Lott et al. [2005] also observed subjective non-response in individuals with the 9/9 genotype ( $n = 8$ ) in a recent study of the effects of the 3' VNTR in *SLC6A3* on the effects of D-amphetamine in 96 adult healthy volunteers. In contrast to these reports, McGough et al. [2006] found no effect of the 10-repeat allele on MPH-induced symptom reduction in ADHD in their prospective study in 81 preschool children with ADHD as did a study by our own group in 82 children from 54 families [van der Meulen et al., 2005]. No effect of the polymorphism in *SLC6A3* (in addition to polymorphisms in *DRD4*, *HTR1B*, *HTR2A*, and *5-HTT*) on treatment response or adverse effects was reported by Zeni et al. [2007] after 1 month of treatment with MPH in 111 children with ADHD, either. A recent study of 106 adults with ADHD also failed to support a role of the 3' UTR polymorphism in response to MPH (both immediate release MPH and longacting OROS MPH) [Mick et al., 2006]. Subjects homozygous for the 10-repeat allele were not distinguishable from heterozygous 9/10 or homozygous 9-repeat allele carriers in their level of symptom reduction or in adverse effects.

In conclusion, currently there is some evidence for both of the frequent *SLC6A3* alleles, the 10- and the 9-repeat allele, being associated with reduced response to MPH, though a number of studies find no effect at all.

A second gene studied for its effect on MPH treatment outcome is *DRD4*, a gene consistently found to be associated with ADHD, which encodes the dopamine receptor D4 [for meta-analysis see Faraone et al., 2005; Li et al., 2006]. Although this protein is not a direct target of MPH, it is an important determinant of dopamine action. In the study by Winsberg and Comings [1999] mentioned above, no effect of a 48-base pair repeat polymorphism in the third exon of *DRD4* (nor of a variant in the *DRD2* gene) on the response to MPH was observed. Seeger et al. [2001], in a prospective study involving 47 children with hyperkinetic disorder (a syndrome classified according to ICD-10 that includes a subgroup of ADHD patients classified by DSM-IV criteria), found the 7R allele of this polymorphism (which is a risk factor for ADHD and associated with a blunted response of the receptor to ligand binding [Asghari et al., 1994, 1995]) to be associated with a reduced response to MPH treatment. This was only observed, however, in interaction with a functional polymorphism of *SLC6A4* (*5-HTT*), the gene encoding the serotonin transporter. A study by Hamarman et al. [2004] found the 7R allele to be associated with a higher dose of MPH needed to achieve treatment response in a sample of 45 children with ADHD. Our own study in 82 children found only a trend towards association of response to MPH in individuals with one or two 7R alleles [van der Meulen et al., 2005], the study by Zeni et al. [2007] found no effect. A second polymorphism in the *DRD4* gene is a 120-bp tandem duplication polymorphism in the 5' flanking region [Seaman et al., 1999]. This polymorphism changes the regulation of the receptor, the longer allele has lower transcriptional activity than the shorter allele [D'Souza et al., 2004]. Minor (non-significant) effects of this polymorphism on response to MPH were reported by McGough et al. [2006] in their prospective study of 81 preschoolers with ADHD.

The norepinephrine transporter might be another target of MPH, and one article reported an association between a single

nucleotide polymorphism (SNP) in the *SLC6A2* gene encoding this transporter and a reduced treatment response to MPH in a sample of 45 children [Yang et al., 2004]. The recent study by McGough et al. [2006], mentioned above, also showed some evidence for the involvement of a genetic variant in the *SNAP25* gene (encoding the synaptosomal-associated protein 25 involved in synaptic vesicle transport) in response to MPH treatment. In addition, a study by Polanczyk et al. [2007] found association of the *ADRA2A* gene encoding the alpha 2A adrenoreceptor with reduction of inattentive symptoms during 3 months of MPH treatment in 106 children and adolescents with ADHD.

For genes that have been studied several times, results often seem inconsistent. The reasons for that are not quite clear, but surely include the mostly small sample sizes used, differences in the design of the studies (e.g., retrospective vs. prospective), MPH dose used and differences in the diagnosis of ADHD and the definition of response to treatment.

To date, almost all pharmacogenetic studies of MPH have focused on treatment in children, exclusively; however, MPH is equally important for the treatment of adults with persistent ADHD of childhood onset. Predictive pharmacogenetic testing in adults may lead to better treatment choices and dosing regimens. We therefore investigated the role of genes with a possible effect on treatment response in children, namely *DRD4*, *SLC6A3*, and *SLC6A2*, in the response of adults with ADHD to MPH treatment in a double-blind, placebo-controlled crossover trial.

## MATERIALS AND METHODS

### Subjects

DNA was obtained from 42 of 45 adults with ADHD, regardless of subtype, who were participating in a double-blind, randomized, placebo-controlled crossover study of immediate release MPH, the results of which have been reported elsewhere [Kooij et al., 2004]. Only subjects with a CGI-severity (CGI-S) equal or greater than 4, and who were impaired in two or more settings were included. Raters were two experienced psychiatrists in adult ADHD, who discussed every patient included in the study.

Subjects with comorbid psychiatric disorders were included unless these disorders had to be treated first or if treatment with MPH was contraindicated. Subjects were prospectively excluded if they had clinically relevant medical conditions, abnormal baseline laboratory values, a history of tic disorders, IQ < 75, organic brain disorders, clinically unstable psychiatric conditions (i.e., suicidal behaviors, psychosis, mania, physical aggression, currently ongoing substance abuse), current use of psychotropics, or prior use of MPH or amphetamines; pregnant or nursing women were also excluded. The study was approved by the local Medical Ethics Committee of the Reinier de Graaf Hospital in Delft. All subjects completed a written informed consent form before inclusion in the study.

### Assessments

Prior to inclusion, patients underwent an extensive clinical assessment for ADHD and comorbidity [Kooij et al., 2004]. The diagnosis of childhood-onset and current ADHD was determined by a psychiatrist's clinical evaluation supplemented by the Dutch version of the DSM-IV ADHD-rating scale for current symptoms during the last 6 months [DuPaul et al., 1998; Kooij et al., 2005].

For a full diagnosis of adult ADHD, subjects had to (1) meet 6 of 9 DSM-IV criteria of inattention and/or hyperactivity/impulsivity for a diagnosis of ADHD in childhood and at least 5



of 9 criteria of one or both domains in adulthood; (2) describe a chronic persisting course of ADHD symptoms from childhood to adulthood; and (3) experience a moderate-to-severe level of impairment attributed to the ADHD symptoms. A cut-off point of 5 of 9 criteria was set for diagnosis of adult ADHD based on the literature and epidemiological data obtained with the same DSM-IV ADHD-Rating Scale [Murphy and Barkley, 1996; Biederman et al., 2000; Kooij et al., 2005; McGough et al., 2005].

Childhood onset of symptoms and impairment (before 12 years of age) was retrospectively assessed by interviewing the patients and their parents or siblings. In addition, partners were interviewed about current symptoms and impairment. Collateral information was available from family members of 39 patients (34 parents and 5 siblings) and of all partners (27). All family members confirmed the diagnosis to a certain extent. The extent varied between "very likely" (24 family members and 24 partners), "likely" (7 family members and 3 partners), and "possible" (8 family members). In addition, in 38 school reports available from 41 patients, comment on behavior supporting the diagnosis in childhood was found.

### Study Design and Outcome

A randomized, placebo-controlled, double-blind cross-over trial comparing immediate-release MPH with placebo was performed. There were two to three-week treatment periods with one week of washout in between. The order of treatment (methylphenidate-placebo or placebo-methylphenidate) was randomized. Medication was prescribed standard every 3–4 hr, resulting in four or five times a day dosing, between 8.00 am and 8.00 pm. Dosing was adjusted to five times a day when rebound occurred. Subjects used a device (Memos) containing compartments for the tablets and a timer in order to dose on time. Study treatment was prescribed at 0.5 mg/kg/day by week 1, followed by 0.75 mg/kg/day by week 2, and up to 1.0 mg/kg/day by week 3. Only the highest dose was considered for measurement of response.

The Dutch self-report-version of the DSM-IV ADHD-rating scale [DuPaul et al., 1998] which is sensitive to drug effects in pediatric [Barkley 2006] and adult [Spencer et al., 1995] samples, was used weekly to assess symptoms of ADHD. For the analyses the scores were first averaged over days of the week and subsequently over all items. Severity of ADHD was assessed weekly with the global severity subscale of the Clinical Global Impression Scale for ADHD (CGI-ADHD; 1 indicates not ill; 7 indicates extremely ill), which is also sensitive to drug effects [NIMH, 1985]. The primary study outcome was clinical response, defined rather strictly a priori as a decrease of at least 2 points on the investigator-based CGI-ADHD severity scale over the total treatment period (3 weeks), as well as a 30% or greater symptom reduction as measured with the self-reported DSM-IV ADHD-rating scale. The two secondary measures of clinical response were a decrease of at least 2 points on the investigator-based CGI-ADHD severity scale, and a 30% or greater symptom reduction as measured with the self-reported DSM-IV ADHD-rating scale, taken separately.

### DNA Isolation and Genotyping

EDTA blood was collected from 42 adult patients. High molecular DNA was isolated by a routine procedure [Miller et al., 1988].

Genotyping was performed for two polymorphisms in the gene encoding the dopamine receptor 4, *DRD4*, the 120-bp tandem insertion/deletion (ins/del) polymorphism upstream of exon 1 [Seaman et al., 1999], and the polymorphism in exon 3 consisting of a variable number of direct imperfect 48-bp

repeats [Van Tol et al., 1992]. The former polymorphism was genotyped using a PCR-based method essentially as described by Seaman et al. [1999]. PCR was on 62.5 ng genomic DNA using 0.4  $\mu$ M of forward primer (5'-GTTGTCTGTCTTTTC-TCAATGTTTCCATTG-3') and 0.4  $\mu$ M reverse primer (5'-GAAGGAGCAGGCACCGTGAGC-3'), 0.25 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen, Breda, The Netherlands) in a PCR buffer containing 10 mM Tris-HCl pH 8.0, 50 mM KCl, 0.1% Triton X-100 (v/v), 0.015% gelatin (w/v), 5% DMSO (v/v) and 1.5 mM MgCl<sub>2</sub>. PCR products were analyzed on a 1.5% agarose gel. The amplification yielded distinct bands at 429 bp (short "S" allele) and 549 bp (long "L" allele). The 48-bp direct repeat polymorphism in *DRD4* was genotyped as described by Lichter et al. [1993]. With 31.25 ng of genomic DNA as template, PCR was performed with 0.4  $\mu$ M forward primer (5'-GCGACTACGTGGTCTACTCG-3') and reverse primer (5'-AGGACCCTCATGGCCTTG-3'), 0.25 mM dNTPs, 0.11 mM 7-deaza GTP (Amersham Biosciences, Roosendaal, The Netherlands), 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands), 60 mM Tris-HCl (pH 8.5), 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10% DMSO (v/v) and 2 mM MgCl<sub>2</sub>. Analysis of the PCR products on a 1.5% agarose gel showed bands at 378 bp (2 repeats), 426 bp (3 repeats), 474 bp (4 repeats), 522 bp (5 repeats), 570 bp (6 repeats), 618 bp (7 repeats), or 666 bp (8 repeats).

The 40-bp VNTR in the 3' untranslated region of the *SLC6A3* gene encoding the dopamine transporter was genotyped as described by Michelhaugh et al. [2001] and Vandenberg et al. [1992]. Genomic DNA (62.5 ng) was amplified with 0.4  $\mu$ M of forward primer (5'-TGTGGTGTAGGGACGGCTGAGAG-3') and reverse primer (5'-CCTTGAGCCGTGACCTCCAGGAA-3') with 0.25 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen) in a PCR buffer containing 60 mM Tris-HCl (pH 8.5), 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10% DMSO (v/v) and 3.5 mM MgCl<sub>2</sub>. Analysis of PCR products was on a 2% agarose gel, producing bands at 443 bp (9 repeats), 483 bp (10 repeats), or 523 bp (11 repeats).

The 4-bp insertion/deletion polymorphism [NETpPR; Urwin et al., 2002] in the promoter region of the *SLC6A2* gene coding for the norepinephrine transporter was analyzed by fragment analysis, using an ABI3100 Genetic Analyser (Applied Biosystems). For the amplification of a PCR fragment containing the variation from 62.5 ng of genomic DNA, a 3-primer system was used with 0.04  $\mu$ M forward primer carrying a universal tail (5'-GGGACACCGCTGATCGTTAGCAGTGTAAATATATGCC-TATGTGCC-3', the universal tail is underlined), 0.4  $\mu$ M reverse primer (5'-GGAGGAGAGGTTTGCCTAC-3'), and 0.36  $\mu$ M of either a 6FAM-, VIC-, or NED-labeled universal primer. In this assay, one of three possible universal primers of different length and labeled with a different fluorescent dye was used for each sample (5'-6FAM-GGGACACCGCTGATCGTTA-3', 5'-VIC-ACGGGACACCGCTGATCGTTA-3', 5'-NED-GACGGGACACCGCTGATCGTTA-3'; stuffer-sequence is underlined). The PCR also contained 0.25 mM dNTP and 1 U Taq DNA polymerase (Invitrogen) in 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1% Triton X-100 (v/v), and 0.15% gelatin (w/v). PCR products containing three different fluorescent labels were pooled for further analysis. The pooling ratio for the fluorescent PCR products containing 6FAM, VIC, and NED was 1:1:1. Genotyping was performed using 1.5  $\mu$ l of the pooled PCR product together with 9.7  $\mu$ l formamide and 0.3  $\mu$ l GeneScan-500 Liz Size Standard™ (Applied Biosystems) on an ABI3100 Genetic Analyzer according to the manufacturer's protocol (Applied Biosystems). Analysis of the PCR products with Genemapper software (Applied Biosystems) showed fragments at 476 bp and 480 bp (using universal 6FAM-labeled primer); 478 bp and 482 bp (using universal VIC-labeled primer), and at 479 bp and 483 bp (using universal NED-labeled primer).

The cycling conditions for most of the PCR assays were similar, starting with 5 min at 92°C, followed by 35 cycles of 1 min at 92°C, 1 min at the optimized annealing temperature (58°C for the *DRD4* 120-bp tandem duplication, the 40-bp VNTR in *SLC6A3*, and the NETpPR polymorphism in *SLC6A2*), and 1 min 72°C (2 min in case of NETpPR), then followed by an extra 5 min 72°C. The cycling conditions for the 48-bp repeat polymorphism in *DRD4* started with 10 min at 92°C, followed by 40 cycles of 45 sec at 95°C, 45 sec at 54°C, and 75 sec at 72°C, with a final extension for 10 min at 72°C. The amplifications were performed in a PTC-200 Multicycler (MJ-Research via Biozym, Landgraaf, The Netherlands).

### Statistics

Responder and non-responder characteristics were compared using Pearson's Chi-square test, Fisher's exact test or the Mann-Whitney *U*-test, where appropriate.

The genotype frequencies were tested for Hardy–Weinberg equilibrium by Chi-square analysis. In the main analysis, data were stratified by treatment (placebo or MPH). Only the results for MPH treatment are presented. Differences in genotype frequencies between responders and non-responders were analyzed using Chi-square tests. When cell frequencies dropped below five, Fisher's exact test was applied. Odds ratios and 95% confidence intervals were generated through logistic regression analysis. Based on the results of the stratified analysis, a random effects logistic regression model was used to formally test for statistical interaction between the genetic variants of the polymorphisms and treatment response while taking the crossover design into account. Analysis took place on an intention-to-treat principle.

All *P*-values were two-tailed and *P*-values below 0.05 were considered significant. Since this was considered a purely explorative study, no correction for multiple testing was applied. Analyses were performed in Stata 8.0 (StataCorp. 2003. Stata Statistical Software: Release 8.0 College Station, TX: StataCorp LP).

### RESULTS

All participants were of Caucasian ethnic origin. None had been treated with stimulants before. Median IQ was 101.5 (range 76–142); the percentage of participants with primary, secondary, and tertiary educational level was 54.8%, 33.3%, and 11.9%, respectively. Mean baseline scores were 1.67 (SD  $\pm$  0.52) for the self-report DSM-IV ADHD-rating

scale and 5.57 (SD  $\pm$  0.91) for the investigator-reported CGI severity score; after treatment, these scores were 1.26 (SD  $\pm$  0.71) and 4.41 (SD  $\pm$  1.64), respectively. Most participants (76.2%) were diagnosed with lifetime comorbid disorders (median 2, range 1–9), the main ones being mood disorders (52.4%), anxiety disorders (45.2%), substance use disorders (SUD; 40.5%), eating disorders (7.1%), and personality disorders (33.3%). Sixteen participants (38.1%) responded to treatment (responders), as defined by a decrease of at least 2 points on the investigator-based CGI-ADHD severity scale over the total treatment period (3 weeks), plus a 30% symptom reduction or more as measured by the self-report DSM-IV ADHD-rating scale. Twenty-six (61.9%) patients did not show a clinical response to MPH and were labeled non-responders. MPH response rates were 51% and 42% for the CGI-S and ADHD-RS, respectively.

Mean MPH doses at endpoint were not different between responders and non-responders (0.94 mg/kg vs. 0.93 mg/kg). The characteristics of both groups are given in Table I. Non-responders had significantly more lifetime comorbid disorders (*P* = 0.009). The number of patients with lifetime SUD was borderline significantly more frequent in the group of non-responders (*P* = 0.05).

The frequency of genotypes detected in the total group is listed in Table II. These were comparable to those found in other studies of individuals of Caucasian ethnic origin (ALFRED database: <http://alfred.med.yale.edu/alfred/index.asp>) [Bakker et al., 2005]. Testing for Hardy–Weinberg equilibrium did not reveal a significant deviation from expected values (*DRD4* (120-bp ins/del): *P* = 0.758; *DRD4* (48-bp repeat): *P* = 0.155; *SLC6A2*: *P* = 0.758; *SLC6A3*: *P* = 0.255).

The results of the stratified analysis of response to MPH are presented in Table II. No association was found between treatment response and the *DRD4* 120-bp ins/del or the 48-bp repeat in this gene. For the latter polymorphism, the analysis was performed for association of carriership of the 7R (the “ADHD risk allele”) and/or the 2R allele compared to a group of all other genotypes only, due to the large number of observed alleles. The 2R allele was included because it seems to be derived directly from the 7R allele by recombination, it is also associated with a blunted response of the receptor to ligand binding, and is a risk factor for ADHD, at least in individuals with an Asian ethnic background [Wang et al., 2004; Leung et al., 2005].

The NETpPR polymorphism in *SLC6A2* was also not associated with the response to MPH.

TABLE I. Demographics of the Study Population

Variable	Total N (%)	Non-responders N (%)	Responders N (%)	Statistics <sup>a</sup> <i>P</i> -value
Age, median (range)	42 (100)	26 (61.9)	16 (38.1)	
Sex: Male N (%)	42.5 (20.1–55.7)	43.0 (20.1–55.7)	42.0 (20.1–52.1)	n.s.
ADHD combined type (%)	23 (54.8)	16 (61.5)	7 (43.8)	n.s.
Baseline ADHD-RS score, mean (SD)	40 (95.2)	26 (100)	14 (87.5)	n.s.
Baseline CGI-S score, mean (SD)	1.67 (0.52)	1.64 (0.48)	1.71 (0.60)	n.s.
Total IQ, mean (SD)	5.57 (0.91)	5.65 (0.75)	5.44 (1.15)	n.s.
Any lifetime comorbid disorder, N (%)	101.83 (18.24)	99.15 (18.94)	106.19 (16.69)	n.s.
Number of lifetime comorbid disorders, median (range)	32 (76.5)	18 (69.2)	14 (87.5)	n.s.
Type of lifetime comorbid disorders, N (%)	2 (1–9)	3 (1–8)	1 (1–9)	<i>P</i> = 0.009
Mood disorders	22 (52.4)	14 (53.9)	8 (50.0)	n.s.
Anxiety disorders	19 (45.2)	12 (46.2)	7 (43.8)	n.s.
Substance use disorders	17 (40.5)	14 (53.8)	3 (18.8)	<i>P</i> = 0.05
Eating disorders	3 (7.1)	1 (3.8)	2 (12.5)	n.s.

ADHD-RS: DSM-IV ADHD rating scale; CGI-S: Clinical Global Impression, Severity scale.

<sup>a</sup>Responder and non-responder characteristics were compared using Pearson's Chi-square test, Fisher's exact test or the Mann–Whitney *U*-test, where appropriate. n.s. = non-significant.

TABLE II. Genotype and Response to Methylphenidate

Gene (polymorphism)	Allelotype	Total number of alleles (%)	Non-responders number of alleles (%)	Responders number of alleles (%)	OR (95% CI)
<i>SLC6A3 (DAT1)</i> (VNTR)	10	59 (70.2)	39 (66.1)	20 (33.9)	1 (—)
	9, 11	25 (29.8)	13 (52.0)	12 (48.0)	1.8 (0.6–5.2)
	Genotype	Number of subjects (%)	Number of subjects (%)	Number of subjects (%)	
	10/10	18 (42.9)	14 (77.8)	4 (22.2)	1 (—)
	10/11, 10/9	23 (54.8)	11 (47.8)	12 (52.2)	3.8 (1.0–15.2)
	9/9	1 (2.4)	1 (100.0)	0 (0.0)	(—) Observation deleted
<i>DRD4</i> ins/del	Allelotype	Number of alleles(%)	Number of alleles (%)	Number of alleles (%)	
	L	63 (75.0)	38 (60.3)	25 (39.7)	1 (—)
	S	21 (25.0)	14 (66.7)	7 (33.3)	0.8 (0.2–2.4)
	Genotype	Number of subjects (%)	Number of subjects (%)	Number of subjects (%)	
	L/L	24 (57.1)	14 (58.3)	10 (41.7)	1 (—)
	L/S	15 (35.7)	10 (66.7)	5 (33.3)	0.7 (0.2–2.7)
<i>DRD4</i> VNTR	S/S	3 (7.1)	2 (66.7)	1 (33.3)	0.7 (0.1–8.8)
	Allelotype	Number of alleles (%)	Number of alleles (%)	Number of alleles (%)	
	Alleles other than 7 or 2	57 (67.9)	36 (63.2)	21 (36.8)	1 (—)
	7, 2	27 (32.1)	16 (59.3)	11 (40.7)	1.2 (0.4–3.3)
	Allelotype	Number of alleles (%)	Number of alleles (%)	Number of alleles (%)	
	2	63 (75.0)	37 (58.7)	26 (41.3)	1 (—)
<i>SLC6A2 (NET)</i> ins/del	1	21 (25.0)	15 (71.4)	6 (28.6)	0.6 (0.2–1.8)
	Genotype	Number of subjects (%)	Number of subjects (%)	Number of subjects (%)	
	2/2	24 (57.1)	13 (54.2)	11 (45.8)	1 (—)
	1/2	15 (35.7)	11 (73.3)	4 (26.7)	0.4 (0.1–1.7)
	1/1	3 (7.1)	2 (66.7)	1 (33.3)	0.6 (0.0–7.4)

The VNTR in *SLC6A3* was almost significantly associated with treatment response when comparing the group of heterozygotes for the allele with 10-repeat units and the 10/10 homozygotes (the 10-repeat allele is the “ADHD risk allele”). The single 9/9 homozygous patient was removed from the analysis, since according to the publication of Lott et al. [2005] this genotype might have significantly different effects from the heterozygous genotypes containing the 10-repeat allele and should therefore be considered separately. Whereas 52.2% of the participants heterozygous for the 10-repeat allele showed a significant improvement in their condition with MPH treatment (12 out of 23), only 22.2% of the 10/10 homozygous individuals achieved a comparable response (4 out of 18) (OR

3.8; 95% CI 1.0–15.2). When we looked at the two measures of response (CGI-ADHD severity scale and self-report DSM-IV ADHD-rating scale) separately (Table III), we found a statistically significant increased odds ratio for MPH treatment response (OR 5.4; 95% CI 1.4–21.9) in the heterozygous 10-repeat allele carriers in comparison with the 10/10 homozygotes for the self-report data, but not for the investigator-based CGI-ADHD severity scale score (OR 1.6; 95% CI 0.5–5.6). Since SUD was more prevalent in the non-responder group compared to the responders (with borderline significance), we excluded the possibility of bias in the estimation of the association between the VNTR in *SLC6A3* and response to MPH by including SUD as a covariate, although we did not

TABLE III. Treatment Response and *SLC6A3 (DAT1)* Genotype Using Two Rating Scales

Outcome measure	Allele/genotype	Non-responders N (%)	Responders N (%)	OR (95% CI)
ADHD-RS	10	37 (62.7)	22 (37.3)	1 (—)
	9, 11	11 (44.0)	14 (66.0)	2.1 (0.7–6.2)
	10/10	14 (77.8)	4 (22.2)	1 (—)
	10/11, 10/9	9 (39.1)	14 (60.9)	5.4 (1.4–21.9)
	9/9	1 (100.0)	0 (0.0)	(—) Observation deleted
CGI-S	10	30 (50.8)	29 (49.2)	1 (—)
	9, 11	12 (48.0)	13 (52.0)	1.1 (0.4–3.2)
	10/10	10 (55.6)	8 (44.4)	1 (—)
	10/11, 10/9	10 (40.0)	13 (60.0)	1.6 (0.5–5.6)
	9/9	1 (100.0)	0 (0.0)	(—) Observation deleted

ADHD-RS: DSM-IV ADHD rating scale; CGI-S: Clinical Global Impression, Severity scale.



observe an association between the polymorphisms and the presence of SUD. The odds ratio for clinical response to MPH was slightly elevated to 4.6 (95% CI 1.0–20.6), and the odds ratio's for response to MPH using the ADHD-RS and CGI-S changed to 6.4 (95% CI 1.5–31.3) and 1.7 (95% CI 0.5–6.4), respectively.

In line with our findings from the stratified analysis, the random effects logistic regression model showed an increased likelihood of a response to MPH treatment in the heterozygotes compared with the 10/10 homozygotes (data not shown). However, the interaction term was not statistically significant ( $P = 0.101$ ).

## DISCUSSION

This is one of the first pharmacogenetic studies of MPH in adults with ADHD. In spite of adequate dosing of immediate release MPH up to 0.94 mg/kg/day, the response rate to MPH observed was lower than the 78% response rate observed in the most comparable US study using the same design, dosage per day, and outcome measures [Spencer et al., 1995]. Explanations for this difference may be the use of only investigator-based outcome measures by Spencer and coworkers, whereas we used both investigator-based and subject-based ratings (CGI-severity and DSM-IV ADHD rating scale), although this can only partly explain the difference. A possible other explanation may be that, although we used the same dosage per day (~1 mg/kg), we used a four to five times a day dosing regimen, whereas Spencer dosed three times a day, leading to a lower dose per unit time in our study. We further enrolled a larger percentage of patients (41% vs. 29%) with additional psychiatric problems and more impairment on various measures (lifetime comorbidity, personality disorders, prior treatment, IQ, school failure, SES). These patients might show lower response rates than a higher educated population being referred for underachievement that was included in the study by Spencer.

The results of our explorative study suggest that the VNTR in the 3' UTR of the *SLC6A3* gene might be associated with the MPH treatment response. The 10/10 genotype was associated with a lower probability of a significant reduction in ADHD symptoms after 3 weeks of treatment, when compared with genotypes with only a single 10-repeat allele in the stratified analysis as well as in the random effects logistic regression model. In the latter analysis the finding did not reach statistical significance but due to our small sample size and the relative modest interaction effect, this was not surprising. Genetic variants in *DRD4* and *SLC6A2* did not seem to affect the response to MPH treatment in our patients.

Our findings regarding *SLC6A3* support those of earlier studies by Winsberg and Comings [1999]; Roman et al. [2002]; Cheon et al. [2005]; Gilbert et al. [2006], but are in contrast to the results published by Kirley et al. [2003]; Stein et al. [2005]; van der Meulen et al. [2005]; McGough et al. [2006]; Mick et al. [2006]; Zeni et al. [2007]. Although the reason for this discrepancy is not entirely clear (though several of the points mentioned in the introduction might apply), several recent studies have found that the 10-repeat allele is associated with a higher expression and/or activity of the dopamine transporter, both in vitro and in vivo [Heinz et al., 2000], though this is not consistently reported [Krause et al., 2006]. Since therapeutic doses of MPH need to block more than 50% of the dopamine transporters in order to significantly enhance the extracellular dopamine concentration [Fuke et al., 2001; Mill et al., 2002; Volkow et al., 2002; Cheon et al., 2005], the increased availability of the transporter molecule in the brain of individuals with the *SLC6A3* 10/10 genotype could mean that higher doses of MPH are needed to achieve appropriate blockade of the dopamine transporter. This is also suggested

by Cheon et al. [2005] who found higher DAT binding ratios during [ $^{123}$ I]IPT SPECT in 10/10 homozygous children compared to other genotypes and also a better response to MPH in the latter group. Thus, if the dose of MPH is not sufficient in patients with the 10/10 genotype, which might be the case with fixed dosing schedules or if low doses are used because of adverse effects, patients will have a poorer response to treatment than patients with other genotypes. Conversely, if dose requirements for the 10/10 homozygotes are met, the potentially negative effect of the genotype on the clinical response would be averted and 10/10 genotype carriers may even have a more pronounced response to MPH treatment, compared to other genotypes. Although this hypothesis is purely speculative, findings of a recent study by Lott et al. [2005] regarding the effects of the *SLC6A3* 3' VNTR genotype on the response to amphetamine in adult healthy volunteers might add some evidence, since the authors indeed observed non-response in individuals homozygous for the 9/9 genotype ( $n = 8$ ), an effect that might be masked by the 10/10 effect if dosing is inadequate. Different durations of treatment may also (in combination with MPH dose) underlie the contradictory results of *SLC6A3* pharmacogenetic studies, with one or the other allele becoming more important upon long term treatment than in short term treatment. This fits well with suggestions of a role of MPH in downregulating DAT expression [e.g., Cheon et al., 2005]. Interestingly, we found the association of the 10/10 genotype with the response to MPH to be most pronounced when this response was measured by patient self-report rather than with a clinician-completed scale. This aspect of who reports on response measures should be taken into account when comparing research data in the future.

We recognize that the power of our study was limited and that the results should be considered preliminary, until replicated. The small sample size may have led to spurious positive findings (type I error) and may have limited the power to detect differences between groups that are only moderate in size. We did not adjust the  $P$ -value to the number of tests due to power considerations because this could increase the type II error rate too much for this explorative study. The small sample size might explain why we were not able to confirm the earlier findings of an association between *DRD4* and *SLC6A2* and the clinical response to MPH treatment in ADHD in children. However, this study and others suggest that the *SLC6A3* genotype may be associated with the response to MPH in children and adults with ADHD. Future studies are needed to disentangle the differential effects of comorbidity, dosing regimens and duration of treatment on the moderating effects of genotypes on treatment response. Furthermore, future studies should take a more complete look at the candidate genes by taking additional polymorphisms into account and assessing LD structure of a gene. From recent studies we already know that for example other polymorphisms in *SLC6A3* also show association with ADHD [Brookes et al., 2006; Asherson et al., 2007]. These might be good candidates to test for association with MPH response, as well.

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